6-906

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From: Prouty, Rebecca
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Art Unit 1814
308-4000
Serial Number: 08/663,618

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FILE 'USPAT' ENTERED AT 16:11:50 ON 26 JUN 1997
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  *                W E L C O M E   T O   T H E                    *
  *          U. S.   P A T E N T   T E X T   F I L E              *
  * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * * *
=> s chitinase# or chitotriosidase#
           224 CHITINASE#
             0 CHITOTRIOSIDASE#
L1           224 CHITINASE# OR CHITOTRIOSIDASE#
=> s l1(5a)human
        147648 HUMAN
L2           0 L1(5A)HUMAN
=> logoff y
U.S. Patent & Trademark Office LOGOFF AT 16:12:46 ON 26 JUN 1997
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FILE 'HOME' ENTERED AT 16:12:15 ON 26 JUN 1997

=> fil .bec

COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.15       0.15

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       WPIDS' ENTERED AT 16:12:26 ON 26 JUN 1997
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

9 FILES IN THE FILE LIST

=> s chitinase# or chitotriosidase#

FILE 'MEDLINE'
           719 CHITINASE#
            11 CHITOTRIOSIDASE#
L1          726 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'SCISEARCH'
          1496 CHITINASE#
            11 CHITOTRIOSIDASE#
L2         1502 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'LIFESCI'
           730 CHITINASE#
             1 CHITOTRIOSIDASE#
L3          730 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'BIOTECHDS'
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             0 CHITOTRIOSIDASE#
L4          562 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'BIOSIS'
          2105 CHITINASE#
            11 CHITOTRIOSIDASE#
L5         2112 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'EMBASE'
           554 CHITINASE#
            10 CHITOTRIOSIDASE#
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L6          560 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'HCAPLUS'
           2227 CHITINASE#
              5 CHITOTRIOSIDASE#
L7         2228 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'NTIS'
             30 CHITINASE#
              0 CHITOTRIOSIDASE#
L8           30 CHITINASE# OR CHITOTRIOSIDASE#

FILE 'WPIDS'
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              0 CHITOTRIOSIDASE#
L9          198 CHITINASE# OR CHITOTRIOSIDASE#

TOTAL FOR ALL FILES
L10        8648 CHITINASE# OR CHITOTRIOSIDASE#

=> s l10(5a)human

FILE 'MEDLINE'
        5972094 HUMAN
L11          11 L1 (5A)HUMAN

FILE 'SCISEARCH'
         608723 HUMAN
L12          12 L2 (5A)HUMAN

FILE 'LIFESCI'
         196528 HUMAN
L13           5 L3 (5A)HUMAN

FILE 'BIOTECHDS'
          27024 HUMAN
L14           1 L4 (5A)HUMAN

FILE 'BIOSIS'
        3817329 HUMAN
L15          21 L5 (5A)HUMAN

FILE 'EMBASE'
        3076244 HUMAN
L16          10 L6 (5A)HUMAN

FILE 'HCAPLUS'
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        632417 HUMAN
L17          15 L7 (5A)HUMAN

FILE 'NTIS'
          68009 HUMAN
L18            0 L8 (5A)HUMAN

FILE 'WPIDS'
          54247 HUMAN
L19            1 L9 (5A)HUMAN

TOTAL FOR ALL FILES
L20           76 L10(5A) HUMAN

=> s l20 not 1997/py

FILE 'MEDLINE'
          93361 1997/PY
L21            9 L11 NOT 1997/PY

FILE 'SCISEARCH'
        345900 1997/PY
L22           10 L12 NOT 1997/PY

FILE 'LIFESCI'
           7429 1997/PY
L23            5 L13 NOT 1997/PY

FILE 'BIOTECHDS'
           3788 1997/PY
                  (1997/PY)
L24            1 L14 NOT 1997/PY

FILE 'BIOSIS'
        141695 1997/PY
L25           18 L15 NOT 1997/PY

FILE 'EMBASE'
        134720 1997/PY
L26            9 L16 NOT 1997/PY

FILE 'HCAPLUS'
        243573 1997/PY
L27           13 L17 NOT 1997/PY

FILE 'NTIS'
           2677 1997/PY
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L28 0 L18 NOT 1997/PY

FILE 'WPIDS'
281024 1997/PY
L29 1 L19 NOT 1997/PY

TOTAL FOR ALL FILES
L30 66 L20 NOT 1997/PY

=> dup rem l30

PROCESSING COMPLETED FOR L30
L31 22 DUP REM L30 (44 DUPLICATES REMOVED)

=> d 1-

L31 ANSWER 1 OF 22 BIOTECHDS COPYRIGHT 1997 DERWENT INFORMATION LTD
TI New ***human*** ***chitinase*** and related nucleic acid, antibodies, transformed cells, etc.;
for use in drug delivery and controlled release implant; diagnostic DNA probe and DNA primer; gene therapy of protozoon infection, Gaucher disease, multiple sclerosis, etc.
AU Aerts J M F G
AN 97-03694 BIOTECHDS
PI WO 9640940 19 Dec 1996

L31 ANSWER 2 OF 22 HCAPLUS COPYRIGHT 1997 ACS
TI Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family
SO J. Biol. Chem. (1996), 271(32), 19415-19420
CODEN: JBCHA3; ISSN: 0021-9258
AU Hu, Bo; Trinh, Kien; Figueira, William F.; Price, Paul A.
AN 1996:498528 HCAPLUS
DN 125:161536

L31 ANSWER 3 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI Use of a recombinant Coccidioides immitis complement fixation antigen-chitinase in conventional serological assays.
SO Journal of Clinical Microbiology 34 (12). 1996. 3160-3164. ISSN: 0095-1137
AU Johnson S M; Zimmermann C R; Pappagianis D
AN 97:20073 BIOSIS

L31 ANSWER 4 OF 22 HCAPLUS COPYRIGHT 1997 ACS
TI Molecular cloning and characterization of an estrogen-dependent porcine oviductal secretory glycoprotein

SO Biol. Reprod. (1996), 55(6), 1305-1314
CODEN: BIREBV; ISSN: 0006-3363
AU Buhi, W. C.; Alvarez, I. M.; Choi, I.; Cleaver, B. D.; Simmen, F. A.
AN 1997:49045 HCAPLUS
DN 126:129790

L31 ANSWER 5 OF 22 MEDLINE DUPLICATE 2
TI Chitinase levels in guinea pig blood are increased after systemic infection with Aspergillus fumigatus.
SO GLYCOBIOLOGY, (1996 Sep) 6 (6) 627-34.
Journal code: BEL. ISSN: 0959-6658.
AU Overdijk B; Van Steijn G J; Odds F C
AN 97081715 MEDLINE

L31 ANSWER 6 OF 22 MEDLINE DUPLICATE 3
TI Molecular cloning of a third chitinase gene (CHT1) from Candida albicans.
SO YEAST, (1996 Apr) 12 (5) 501-4.
Journal code: YEA. ISSN: 0749-503X.
AU McCreath K J; Specht C A; Liu Y; Robbins P W
AN 96310630 MEDLINE

L31 ANSWER 7 OF 22 HCAPLUS COPYRIGHT 1997 ACS
TI On the stability of human lysosomal enzymes at room temperature in normal and acidified plasma and serum
SO Clin. Chim. Acta (1996), 244(2), 229-35
CODEN: CCATAR; ISSN: 0009-8981
AU Den Tandt, W. R.
AN 1996:76851 HCAPLUS
DN 124:196868

L31 ANSWER 8 OF 22 MEDLINE DUPLICATE 4
TI Cloning of a cDNA encoding ***chitotriosidase*** , a ***human*** ***chitinase*** produced by macrophages.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Nov 3) 270 (44) 26252-6.
Journal code: HIV. ISSN: 0021-9258.
AU Boot R G; Renkema G H; Strijland A; van Zonneveld A J; Aerts J M
AN 96064695 MEDLINE

L31 ANSWER 9 OF 22 MEDLINE DUPLICATE 5
TI ***Chitinase*** activity in ***human*** serum and leukocytes.
SO INFECTION AND IMMUNITY, (1995 Dec) 63 (12) 4770-3.
Journal code: GO7. ISSN: 0019-9567.
AU Escott G M; Adams D J
AN 96071897 MEDLINE

L31 ANSWER 10 OF 22 MEDLINE DUPLICATE 6
TI Purification and characterization of ***human*** ***chitotriosidase*** , a novel member of the chitinase family of proteins.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Feb 3) 270 (5) 2198-202. Journal code: HIV. ISSN: 0021-9258.
AU Renkema G H; Boot R G; Muijsers A O; Donker-Koopman W E; Aerts J M
AN 95138187 MEDLINE

L31 ANSWER 11 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI Possible roles of wall hydrolases in the morphogenesis of Coccidioides immitis.
SO Canadian Journal of Botany 73 (SUPPL. 1 SECT. E-H). 1995. S1132-S1141. ISSN: 0008-4026
AU Cole G T; Pishko E J; Seshan K R
AN 96:476044 BIOSIS

L31 ANSWER 12 OF 22 MEDLINE DUPLICATE 7
TI Differential recognition of microfilarial chitinase, a transmission-blocking vaccine candidate antigen, by sera from patients with Brugian and Bancroftian filariasis.
SO AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE, (1995 Sep) 53 (3) 289-94. Journal code: 3ZQ. ISSN: 0002-9637.
AU Dissanayake S; Perler F B; Xu M; Southworth M W; Yee C K; Wang S; Dreyer G; Watawana L; Kurniawan L; Fuhrman J A; et al
AN 96033016 MEDLINE

L31 ANSWER 13 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI Does serum YKL-40 reflect disease activity in rheumatoid arthritis and osteoarthritis?.
SO 59th National Scientific Meeting of the American College of Rheumatology and the 30th National Scientific Meeting of the Association of Rheumatology Health Professionals, San Francisco, California, USA, October 21-26, 1995. Arthritis & Rheumatism 38 (9 SUPPL.). 1995. S217. ISSN: 0004-3591
AU Johansen J S; Hansen M; Stoltenberg M; Hvolris J; Florescu A; Price P A; Horslev-Petersen K
AN 95:520893 BIOSIS

L31 ANSWER 14 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI A rapid method for the isolation of genomic DNA from Aspergillus fumigatus.
SO Preparative Biochemistry 25 (4). 1995. 171-181. ISSN: 0032-7484
AU Bir N; Paliwal A; Muralidhar K; Reddy P; Sarma P U
AN 96:23117 BIOSIS

L31 ANSWER 15 OF 22 MEDLINE DUPLICATE 8
TI Cloning and expression in Escherichia coli of the nahA gene from Porphyromonas gingivalis indicates that beta-N-acetylhexosaminidase is an outer-membrane-associated lipoprotein.
SO MICROBIOLOGY, (1994 Dec) 140 (Pt 12) 3399-406.
Journal code: BXW. ISSN: 1350-0872.
AU Lovatt A; Roberts I S
AN 95187310 MEDLINE

L31 ANSWER 16 OF 22 MEDLINE DUPLICATE 9
TI ***Human*** serum contains a ***chitinase*** : identification of an enzyme, formerly described as 4-methylumbelliferyl-tetra-N-acetylchitotetraoside hydrolase (MU-TACT hydrolase).
SO GLYCOBIOLOGY, (1994 Dec) 4 (6) 797-803.
Journal code: BEL. ISSN: 0959-6658.
AU Overdijk B; Van Steijn G J
AN 95252690 MEDLINE

L31 ANSWER 17 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI The cloning and sequencing of two separate ***chitinase*** genes from the ***human*** pathogenic fungus Coccidioides immitis.
SO 94th General Meeting of the American Society for Microbiology, Las Vegas, Nevada, USA, May 23-27, 1994. Abstracts of the General Meeting of the American Society for Microbiology 94 (0). 1994. 589. ISSN: 1060-2011
AU Pishko E J; Cole G T
AN 94:333366 BIOSIS

L31 ANSWER 18 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI Paracoccidioides brasiliensis protoplast production by enzymatic treatment.
SO Mycoses 37 (9-10). 1994. 317-323. ISSN: 0933-7407
AU Borba C D M; Meirelles M N S L; Silva A M M D; Oliveira P C D
AN 95:222976 BIOSIS

L31 ANSWER 19 OF 22 SCISEARCH COPYRIGHT 1997 ISI (R) DUPLICATE 10
TI EXPRESSION OF A ***CHITINASE*** -LIKE PROTEIN (C-GP39) IN ***HUMAN*** ARTICULAR-CARTILAGE AND SYNOVIUM
SO ARTHRITIS AND RHEUMATISM, (SEP 1993) Vol. 36, No. 9, Supp. S, pp. S190.
ISSN: 0004-3591.
AU RECKLIES A D (Reprint); BAILLARGEON L; WHITE C
AN 93:640125 SCISEARCH

L31 ANSWER 20 OF 22 MEDLINE DUPLICATE 11
TI The coccidioidal complement fixation and immunodiffusion-complement fixation antigen is a chitinase.

SO INFECTION AND IMMUNITY, (1992 Jul) 60 (7) 2588-92.
Journal code: GO7. ISSN: 0019-9567.
AU Johnson S M; Pappagianis D
AN 92307878 MEDLINE

L31 ANSWER 21 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
TI COMPARATIVE STUDY OF THE PRODUCTION OF DIVERSE ENZYMES FROM 2 STRAINS OF CONIDIOBOLUS-CORONATUS.
SO BOL SOC MEX MICOL 0 (16). 1981 (RECD. 1982). 5-10. CODEN: BSMMDY
AU MIER T; TORIELLO C; CASAMITJANA M; GARCIA MAYNEZ A M; LOPEZ-MARTINEZ R
AN 83:155942 BIOSIS

L31 ANSWER 22 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS DUPLICATE 12
TI CHITINASE ACTIVITY AND SUBSTRATE SPECIFICITY OF 3 BACTERIOLYTIC ENDO-BETA-N-ACETYL EC-3.2.1 MURAMIDASES AND ENDO-BETA-N ACETYL GLUCOSAMINIDASE.
SO ACTA CHEM SCAND 26 (2). 1972 653-660. CODEN: ACSAA4 ISSN: 0001-5393
AU NORD C E; WADSTROM T
AN 73:102980 BIOSIS

=> d ab 1-

L31 ANSWER 1 OF 22 BIOTECHDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AB A new ***human*** ***chitinase*** (EC-3.2.1.14) DNA sequence encodes a protein sequence of 466 or 388 amino acids. Oligonucleotide DNA probes and primers of at least 8 bases hybridizing with the DNA, a peptide of at least 8 amino acids, mimicking chitinase epitopes, and antibodies binding the chitinase, are also new. The proteins are formed by alternative splicing of RNA, and differ significantly only in the C-terminus, with a highly-conserved catalytically active central region. The DNA may be used in gene therapy of infection by a chitin-containing pathogen (e.g. a fungus, protozoon or helminth), and the enzyme may be used in cell cultures prior to implantation, in cosmetics, dental products or foods (e.g. dairy products). The probes, primers and antibodies may be used diagnostically. Elevated chitinase levels are associated with inherited lysosomal lipidosis (e.g. Gaucher disease), visceral leishmaniasis, sarcoidosis, X-linked adrenoleukodystrophy and multiple sclerosis. The enzyme may be used in controlled release implant compositions, and is harmless, non-immunogenic, active at pH 3-8 and up to 50 deg, and stable in the circulation. (58pp)

L31 ANSWER 2 OF 22 HCAPLUS COPYRIGHT 1997 ACS
AB The authors describe the isolation of a novel protein from the

conditioned medium of human articular cartilage chondrocytes in primary culture. This 39-kDa protein has the N-terminal sequence YKL, which the authors have termed YKL-39. The 1434-nucleotide sequence of the YKL-39 cDNA predicts a 385-residue initial translation product and a 364-residue mature YKL-39. The amino acid sequence of YKL-39 is most closely related to YKL-40, followed by macrophage chitotriosidase, oviductal glycoprotein, and macrophage YM-1. All five proteins share significant sequence identity with bacterial chitinases and have the probable structure of an (.alpha..beta.)8 barrel. YKL-39 lacks the active site glutamate, which is essential for the activity of chitinases, and as expected has no chitinase activity. The highest level of YKL-39 mRNA expression is seen in chondrocytes, followed by synoviocytes, lung, and heart. YKL-39 accounts for 4% of the protein in chondrocyte-conditioned medium, prostromelysin accounts for 17%, and YKL-40 accounts for 33%. In contrast to YKL-40, YKL-39 is not a glycoprotein and does not bind to heparin.

L31 ANSWER 3 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS

AB The coccidioidal complement fixation (CF) antigen has been cloned previously, and the fusion protein has been expressed in Escherichia coli. The recombinant CF (rCF) antigen was affinity purified by adsorption-desorption to chitin, and its reactivity was studied by using sera containing coccidioidal antibodies. The affinity-purified rCF antigen formed a line of identity with an immunodiffusion (ID) CF reference antigen (coccidioidin) derived from mycelial-phase Coccidioides immitis and was reactive with human, canine, and equine sera containing coccidioidal antibody. The affinity-purified rCF antigen yielded no detectable reaction with Blastomyces or Histoplasma antiserum by ID. The affinity-purified rCF antigen fixed complement with positive human sera and, even when used at lower concentrations, yielded titers comparable to those obtained with the coccidioidin. The reactivity of the affinity-purified rCF antigen was further evaluated by enzyme immunoassay, in which it manifested good sensitivity (96.9%) and specificity (100%) when evaluated with 43 human patients' sera. Thus, the affinity-purified rCF antigen has yielded reactions comparable to those of crude coccidioidal antigens in conventional CF, IDCF, and enzyme immunoassay.

L31 ANSWER 4 OF 22 HCAPLUS COPYRIGHT 1997 ACS

AB A family of estrogen-dependent porcine oviductal secretory glycoproteins (POSPs) that exhibit structural similarities are synthesized and secreted into the oviductal lumen at proestrus, estrus, and metestrus. The objectives of this study were to clone the POSP cDNA, obtain the full-length cDNA and protein sequence, examine tissue specificity and species distribution, characterize its regulation, and establish its identity by comparison to other

known protein, RNA, or DNA sequences. A full-length cDNA of 2022 base pairs was obtained with an open reading frame of 1581 nucleotides, coding for a deduced protein of 527 amino acids (57 970 Mr). The deduced protein contained three potential N-glycosylation sites, a consensus heparin-binding site, and potential O-glycosylation sites. Amino acid anal. of POSP-E3 confirmed the presence of a 21-amino acid signal sequence. Northern blot anal. revealed an oviduct-specific mRNA species of 2.25 kb in the infundibulum (INF), ampulla (A), and isthmus (I). An mRNA of similar size was detected in the oviduct of the sheep, cow, and rabbit, and one of slightly greater size (2.8 kb) in the mouse and hamster oviduct but not in the horse or alligator oviduct. Dot blot anal. indicated that steady-state levels of POSP mRNA were significantly greater in the A than in the INF or 1 regardless of day of the estrous cycle and were greater on Day 0 (estrus) regardless of location. Further, steady-state mRNA levels were significantly increased on Days 0 and 1, declining rapidly to Day 2 through Day 15 of the estrous cycle. Steady-state POSP mRNA levels were significantly greater in ovariectomized gilts treated with estradiol valerate than those treated with other steroid regimens, vehicle, or no treatment (Control), consistent with estrogen control of mRNA expression. The POSP protein exhibited significant identity to oviductal glycoproteins from the baboon, cow, hamster, ***human*** , mouse, and several ***chitinases*** . POSP joins a growing subfamily of the chitinase gene family that lacks chitinase enzymic activity.

L31 ANSWER 5 OF 22 MEDLINE DUPLICATE 2

AB The presence of ***chitinase*** activity in ***human*** serum has recently been described by us. On that occasion we speculated on the possible role of mammalian chitinases as a defense mechanism against chitin-containing pathogens. The results of the present study substantiate our hypothesis. We demonstrate and partially characterize the chitinase activities that are present in plasma of guinea pigs and in homogenates of A.fumigatus with the aid of the substrates MU-[GlcNAc]2,3 and also with glycol [3H]chitin. Upon infection with A.fumigatus the serum chitinase activity levels in the circulation of pathogen-free guinea pigs increased in a time-dependent manner. The increase was also dependent on the size of the infecting fungal inoculum. Antifungal treatment diminished the increases. The increased chitinase activity was of guinea pig origin. The activity of beta-hexosaminidase showed a very slight increase subsequent to the infection. The activities of three other enzymes of lysosomal origin (alpha-mannosidase, beta-galactosidase and beta-glucosidase) did not increase.

L31 ANSWER 6 OF 22 MEDLINE DUPLICATE 3

AB Here we report the complete nucleotide sequence of a third ***chitinase*** gene (CHT1) from the dimorphic ***human*** pathogen Candida albicans. The deduced amino acid (aa) sequence of Cht1 consists of 416 aa and displays 36% protein sequence similarity to chitinases Cht2 and Cht3, from C. albicans. Interestingly the domain structure of Cht1 is truncated when compared to the other chitinases of C. albicans and lacks a Ser/Thr-rich region.

L31 ANSWER 7 OF 22 HCAPLUS COPYRIGHT 1997 ACS
AB Stability of lysosomal enzymes in human plasma and serum has been examd. in previous studies. These studies have generally been done either at 37.degree.C or at 4 and -20.degree.C. Clin. samples are often kept at room temp. before they arrive in the lab. for the purpose of diagnosis of lysosomal storage diseases. Because of previous repeated evidence that lysosomal enzymes are more stable on conservation if plasma is acidified (10.mu.l of 5 mol/l acetic acid added to 0.9 mL plasma or serum according to Den Tandt, W.R. et al, J. Lab. Clin. Med., 1974, 83:337-346), we have systematically compared the stability in acidified plasma and serum vs. non-acidified samples kept at room temp. for up to 48 h. The following enzymes were examd.: .beta.-D-galactosidase (E.C. 3.2.1.23), .alpha.-D-galactosidase (E.C. 3.2.1.22), .alpha.-L-iduronidase (E.C. 3.2.1.76), .beta.-D-glucosidase (E.C. 3.2.1.21), .alpha.-D-glucosidase (E.C. 3.2.1.20), .alpha.-D-mannosidase (E.C. 3.2.1.24), .beta.-D-glucuronidase (E.C. 3.2.1.31), N-acetyl-.beta.-D-hexosaminidase (E.C. 3.2.1.52), .alpha.-L-fucosidase (E.C. 3.2.1.51), .beta.-D-mannosidase (E.C. 3.2.1.25), N-acetyl-.alpha.-D-glucosaminidase (E.C.3.2.1.50), methylumbelliferyl-tetra-N-acetyl-.beta.-D-chitotetraoside (MU-TACT) hydrolase (E.C. 3.2.1.14), N-acetyl-.alpha.-D-galactosaminidase (E.C. 3.2.1.49) and N-acetyl-.beta.-D-hexosaminidase A (hexosaminidase A) (E.C. 3.2.1.52).

L31 ANSWER 8 OF 22 MEDLINE DUPLICATE 4
AB We have recently observed that chitotriosidase, a chitinolytic enzyme, is secreted by activated human macrophages and is markedly elevated in plasma of Gaucher disease patients (Hollak, C. E. M., van Weely, S., van Oers, M. H. J., and Aerts, J. M. F. G. (1994) J. Clin. Invest. 93, 1288-1292). Here, we report on the cloning of the corresponding cDNA. The nucleotide sequence of the cloned cDNA predicts a protein with amino acid sequences identical to those established for purified chitotriosidase. Secretion of active chitotriosidase was obtained after transient transfection of COS-1 cells with the cloned cDNA, confirming its identity as chitotriosidase cDNA. Chitotriosidase contains several regions with high homology to those present in chitinases from different species belonging to family 18 of glycosyl hydrolases. Northern blot

analysis shows that expression of chitotriosidase mRNA occurs only at a late stage of differentiation of monocytes to activated macrophages in culture. Our results show that, in contrast to previous beliefs, ***human*** macrophages can synthesize a functional ***chitinase*** , a highly conserved enzyme with a strongly regulated expression. This enzyme may play a role in the degradation of chitin-containing pathogens and can be used as a marker for specific disease states.

L31 ANSWER 9 OF 22 MEDLINE DUPLICATE 5

AB Using colloidal [3H] chitin as a substrate, we provide the first demonstration of a ***chitinase*** in ***human*** leukocytes; chitinolytic activity in whole and disrupted leukocyte preparations (approximately 0.6 and 5.5 nmol of N-acetylglucosamine [GlcNAc] released min-1 mg of protein-1, respectively) was partially inhibited by the specific chitinase inhibitor allosamidin (9 microM). Following fractionation of the leukocytes, much higher levels of chitinase activity were detected in granulocyte-rich homogenates (approximately 7.2 nmol of GlcNAc released min-1 mg of protein-1) than in lymphocyte- and monocyte-rich homogenates (approximately 0.22 and 0.26 nmol of GlcNAc released min-1 mg of protein-1, respectively). Low levels of ***chitinase*** activity were detected in ***human*** serum (approximately 4 pmol of GlcNAc released min-1 mg of protein-1). Chitinolytic activity in granulocyte-rich homogenates and serum was partially inhibited by allosamidin (9 microM). Proteins with chitinolytic activities (approximate molecular masses, 48 and 56 kDa) distinct from lysozyme (14.3 kDa) were detected on polyacrylamide gels following the electrophoresis of ***human*** granulocyte-rich preparations. ***Chitinase*** activity, detected consistently in serum and leukocytes from all human volunteers investigated, may contribute to the protection of the host by cleaving chitin in the cell walls of fungal pathogens.

L31 ANSWER 10 OF 22 MEDLINE DUPLICATE 6

AB Recently we noted (Hollak, C.E.M., van Weely, S., van Oers, M.H.J., and Aerts, J.M.F.G. (1994) J. Clin. Invest. 93, 1288-1292) that the clinical manifestation of Gaucher disease is associated with a several hundred-fold increase in chitotriosidase activity in plasma. We report on the purification and characterization of the protein. Two major isoforms of chitotriosidase with isoelectric points of 7.2 and 8.0 and molecular masses of 50 and 39 kDa, respectively, were purified from the spleen of a Gaucher patient. The N-terminal amino acid sequence of the two forms proved to be identical. An antiserum raised against the purified 39-kDa chitotriosidase precipitated all isozymes. Chitotriosidase activity was earlier found to be completely absent in some individuals. These findings in combination

suggest that a single gene may encode the different isoforms of chitotriosidase. Both the N-terminal sequence and an internal sequence chitotriosidase proved to be homologous to sequences in proteins that are members of the chitinase family (Hakala, B.E., White,C., and Recklies, A.D. (1993) J. Biol. Chem. 268, 25803-25810). The ***human*** ***chitotriosidase*** described here showed chitinolytic activity toward artificial substrates as well as chitin and may therefore be considered to be a chitinase.

L31 ANSWER 11 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS
AB We have used the human respiratory pathogen, Coccidioides immitis, as an experimental model to explore possible interrelationships of wall-associated hydrolases, cell growth, and reproduction. Preliminary evidence has been presented that suggests that certain wall hydrolases (glucanase, chitinase) may play key roles in cell development in this systemic pathogen. Initial differentiation of the parasitic cells from cylindrical arthroconidia involves a period of isotropic growth and results in formation of a multinucleate spherule (approximately 60 mu diameter). An endo-1,3-beta-glucanase that may participate in this diametric growth phase has been isolated. Two distinct chitinase genes (cts1, cts2) have been isolated from C. immitis and shown to be members of different classes of this wall hydrolase. The class I chitinase (CTS2) demonstrates homology to a reported endochitinase of Saccharomyces cerevisiae that has been shown to be essential for yeast daughter cell release. CTS2 may play a pivotal role in isotropic growth, as well as differentiation and release of endospores from maternal spherules. In the absence of specific gene disruption and transformation experiments, these data are still circumstantial evidence for the functions of wall hydrolases in C. immitis development. However, we suggest our results provide further support for the concept that wall hydrolases represent rational molecular targets for future development of novel antifungal agents.

L31 ANSWER 12 OF 22 MEDLINE DUPLICATE 7
AB We examined the reactivity of ***human*** sera with recombinant microfilarial ***chitinase*** and with the antigenic determinant on the native parasite molecule identified by monoclonal antibody (MAb) MF1. In Brugian filariasis, the MF1 epitope is preferentially recognized by residents of endemic areas who remain amicrofilaremic and asymptomatic despite lifelong exposure to filarial worms. Reactivity with filarial chitinase and its MF1 epitope inversely correlates with microfilaremia levels in Bancroftian filariasis and is associated with a prolonged amicrofilaremic state following a single course of treatment with diethylcarbamazine. Chitinase does not appear to be a target of human antibodies that promote the

adherence of cells to microfilariae, even though MAb MF1 itself promotes antibody-dependent, cell-mediated cytotoxic (ADCC) reactions that kill microfilariae in vitro. Such ADCC reactions are most often mediated by sera from amicrofilaremic patients with chronic elephantiasis that contain low or undetectable levels of IgG antibodies to chitinase. In contrast, antibodies to the MF1 epitope on this microfilarial stage-specific antigen are mostly present in amicrofilaremic donors without clinical lymphatic disease. These observations indicate that antibodies to the MF1 epitope of microfilarial chitinase reflect some degree of immune resistance to microfilaremia in a subgroup of patients with asymptomatic lymphatic filariasis. The amicrofilaremic state of individuals with chronic lymphatic disease appears to be mediated by reactivity to a different parasite antigen(s).

L31 ANSWER 13 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS

L31 ANSWER 14 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS

AB A majority of Aspergillus induced diseases are reported to be caused by Aspergillus fumigatus. In immunocompromized and post transplant cases it can lead to invasive aspergillosis. Due to this the molecular fingerprinting of aspergillus isolates by RFLP analysis and development of DNA diagnostic probes are gaining importance. Different methodologies are being adopted for extraction of the genomic DNA from fungus. The existing procedures for isolation of DNA are time consuming and range from several hours to few days. The most difficult step in the isolation of DNA from aspergillus species is to disrupt the tough chitin rich cell wall without causing damage to genomic DNA. We report here a rapid method for extraction of genomic DNA based on the cleavage of chitin with chitinase. The subsequent modification steps included are lysis and microwave treatment. The chromosomal DNA obtained by this procedure is 1.5-2.0 mu-g per mg of wet weight of mycelia and is observed to be minimally sheared It is pure enough for restriction analysis and for use in the PCR to detect the gene coding for 18 kDa allergen which has been identified in our laboratory using western blot analysis with human patient sera.

L31 ANSWER 15 OF 22 MEDLINE DUPLICATE 8

AB Porphyromonas gingivalis has been implicated in human periodontal diseases. It expresses a number of exoglycosidase enzymes capable of hydrolysing host proteoglycan residues. As a first stage to explore the role of these enzymes in periodontal tissue damage, the nahA gene of P. gingivalis W83, which encodes beta-N-acetylhexosaminidase (beta-Nahase), was cloned. The gene was expressed poorly in Escherichia coli, but increased expression was achieved by cloning the nahA gene downstream of the tac promoter. Southern blot analysis revealed that nahA was present as a single copy, and it was found in

all the other P. gingivalis strains tested. In contrast, sequences homologous to nahA were not detected in either P. endodontalis or P. asaccharolytica. The nahA gene was 2331 bp long and encoded a beta-Nahase enzyme of 777 amino acids with a predicted molecular mass of 87 kDa. A characteristic signal peptide for an acylated lipoprotein was present at the amino-terminus, suggesting that the mature beta-Nahase is a lipoprotein. The predicted amino acid sequence of the P. gingivalis beta-Nahase shared homology with the catalytic domains of the ***human*** beta-Nahase enzyme and the ***chitinase*** of Vibrio harveyi, suggesting a common catalytic mechanism.

L31 ANSWER 16 OF 22 MEDLINE DUPLICATE 9

AB Since 1988 an endoglucosaminidase, provisionally named MU-TACT hydrolase, has been known that hydrolyses the artificial substrate 4-methylumbelliferyl-tetra-N-acetyl-chitotetraoside (MU-[GlcNAc]4, where GlcNAc is N-acetylglucosamine). The biological function of the enzyme was unknown. In this paper evidence is presented showing that this endoglucosaminidase from ***human*** serum is in fact a ***chitinase*** that is different from lysozyme. The facts sustaining this finding are: (i) the identification of the products formed from MU-[GlcNAc]3 and [GlcNAc]2;and [GlcNAc]3; (ii) chitin and ethylene glycolchitin can be degraded by the enzyme; (iii) the chitinase inhibitor allosamidin also inhibits the action of MU-TACT hydrolase from human serum; (iv) no hydrolysis of the lysozyme substrate Micrococcus lysodeikticus. The enzyme also occurs in rat liver. It was demonstrated that upon Percoll density gradient centrifugation the enzyme from this tissue distributed parallel to the lysosomal marker enzymes beta-N-acetylhexosaminidase and beta-galactosidase, indicating a lysosomal localization for this enzyme. It is proposed that the enzyme functions in the hydrolysis of chitin, to which mammals are frequently exposed during infection by pathogens.

L31 ANSWER 17 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS

L31 ANSWER 18 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS

AB The action of the enzymes novozym 234, chitinase and zymolyase 20T on the yeast-like cells of Paracoccidioides brasiliensis was studied in an attempt to obtain protoplast release. Three enzyme systems were used: the first consisted of novozym 234 and chitinase plus 0.2 M phosphate buffer, 0.9 M sorbitol and 0.5 M sodium thioglycolate; the second consisted of novozym 234, chitinase, zymolyase 20T, buffer and osmotic stabilizer, with no sodium thioglycolate; the third consisted of the same enzymes as used in the second system but at twice the concentration, plus buffer and osmotic stabilizer. Protoplasts were only released from 72-h-old cells cultured on solid peptone-yeast

extract-glucose medium (PYG) treated with the third enzyme system. Sodium thioglycolate used as pretreatment favoured protoplast release but had no such action when added to the enzyme solution, possibly by altering the activity of the enzymes, novozym 234 in particular. The osmotic stabilizer used, 0.9 M sorbitol, was probably one of the factors, in addition to the enzymes, responsible for the cytoplasmic changes observed by transmission electron microscopy in yeast phase cells and in their protoplasts.

L31 ANSWER 19 OF 22 SCISEARCH COPYRIGHT 1997 ISI (R) DUPLICATE 10

L31 ANSWER 20 OF 22 MEDLINE DUPLICATE 11

AB Culture filtrates and autolysates of Coccidioides immitis have provided suitable crude antigens for the serodiagnosis and prognosis of coccidioidomycosis. One of these, a heat-labile antigen which participates in the immunodiffusion reaction corresponding to the complement fixation reaction (IDCF), has been characterized as a 110-kDa native protein that, when subjected to reducing conditions and heat, yields a 48-kDa component. The present report provides serologic and biochemical evidence that this antigen is a chitinase. This chitinase, isolated from 48-h culture filtrate of the spherule-endospore-phase C. immitis by affinity adsorption to chitin, formed a line of identity with the IDCF reference antigen and participated in the complement fixation reaction with human serum. It lost its enzymatic as well as antigenic activity when heated, but when not heated it retained its enzymatic activity even when precipitated with coccidiodal antibody present in ***human*** serum. This ***chitinase*** represents a significant serodiagnostic substance and may be important in the morphogenesis of C. immitis.

L31 ANSWER 21 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS

AB The production of different enzymes of 2 strains of C. coronatus, 1 isolated from insects and another from a ***human*** case of rhinoentomophthoromycosis, was observed. ***Chitinase*** , protease, hemolysin, DNase and lipase were studied. The enzymes were present in both strains with the exception of chitinase; there was no chitinolytic activity present in these strains. The velocity of growth and diameter of the colony were always greater from the insect strain.

L31 ANSWER 22 OF 22 BIOSIS COPYRIGHT 1997 BIOSIS DUPLICATE 12

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